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TRENDS IN FOOD SCIENCE & TECHNOLOGY

Viewpoint

Plasma LDL cholesterol lowering by plant phytosterols in a hamster model

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Cardiovascular disease is still the main cause of death in the US. High plasma cholesterol, 51.9% of Americans have cholesterol levels of 200 mg/dl or higher and especially low-density lipoprotein (LDL) cholesterol, and high ratios of LDL to high-density lipoprotein (HDL) cholesterol are risk factors for cardiovascular disease. Plant stanol esters have been shown to be effective in reducing plasma cholesterol. Other phytosterol-like compounds such as oryzanol, a mixture of ferulate esters from rice bran, and tomatine, a glycoalkaloid in green tomatoes, have also shown cholesterol-reducing properties in test animals. Investigations of mechanisms and uses of plant phytosterols are facilitated by animal models that respond to phytosterols, as well as other known cholesterol-lowering agents.

The hypercholesterolemic LDL hamster is used to efficiently screen phytosterols for plasma cholesterol-lowering properties. Hypercholesterolemia is induced in these animals by saturated fat and fish oil, and dietary cholesterol intake similar to that of humans, 0.025–0.05%. This results in rapid LDL hypercholesterolemia. Prolonged feeding of the hypercholesterolemic diet results in aortic fatty streak formation, which can be retarded by the antioxidants alpha-tocopherol or catechin. Known plasma cholesterol-reducing agents such as cholestyramine, soluble dietary fiber, stanol esters, oryzanol, and tomatine reduce total and LDL cholesterol. Gas chromatography/mass spectrometry indicates that tomatine binds cholesterol, but fiber and cholestyramine increase bile acid excretion.

Cardiovascular disease affects about 58 million people, or one-fourth of the US population, and kills about 960,000 people annually (Chronic Diseases and Their Risk Factors, 1999). It is the major cause of death in the US Risk factors include increasing age, gender, heredity, overweight, low physical activity, tobacco smoke, high blood pressure, and diabetes. High serum cholesterol (>240 mg/dl) and high concentrations of low-density lipoprotein cholesterol (LDL-C, >160 mg/dl) are also risk factors for cardiovascular disease (AHA. American Heart Association, 2000). Risk factors such as overweight, physical inactivity, smoking, diabetes, and serum cholesterol can be reduced. Since the 1950s, it has been known that plant sterol mixtures can reduce plasma cholesterol, and recently, the US Food and Drug Administration recognized the effectiveness of these compounds (stanol and sterol esters), by approving health claims for margarines and other foods containing these compounds (Federal Register, 2000).

Fig. 1 shows the structures of cholesterol, β-sitosterol and cycloartenol ferulate. Phytosterols such as β-sitosterol or campesterol are examples of cholesterol-lowering sterols that can be used in margarines. These compounds are commonly found in oils of plant origin, such as soy, corn, peanut, and other food oils. The structures of these phytosterols are closely related to one another and also resemble that of cholesterol (Fig. 1). Phytosterol and stanol esters are more soluble in lipids and are more easily formulated in foods than the free sterol or stanol in lowering serum cholesterol. The phytosterols are thought to displace cholesterol from bile acid micelles and/or co-precipitate cholesterol in the intestinal lumen, thereby limiting its uptake. Cholesterol also serves as the precursor of bile acids, and serum cholesterol is lowered by bile acid binding to resins such as cholestyramine.

Rice bran also contains phytosterols and γ-oryzanol, that lower plasma cholesterol. γ-Oryzanol is a mixture of ferulic acid esters of cycloartenol (Fig. 1), methylene cycloartenol, cycloartanol, and other sterols (Rogers *et al.*, 1993). Ferulate esters of campesterol, and sitostanol and campestanol are found in rice and corn bran, respectively (Norton, 1995). γ-Oryzanol has been reported to reduce plasma cholesterol in hamsters fed hypercholesterolemic (5% coconut oil and 0.1% cholesterol) rodent chow based diets (Rong, Ausman, & Nicolosi *et al.*, 1997).

Experimental protocol

Typically, 10 male golden Syrian Hamsters (Sasco strain, Charles River Laboratories, Wilmington, MA) weighing

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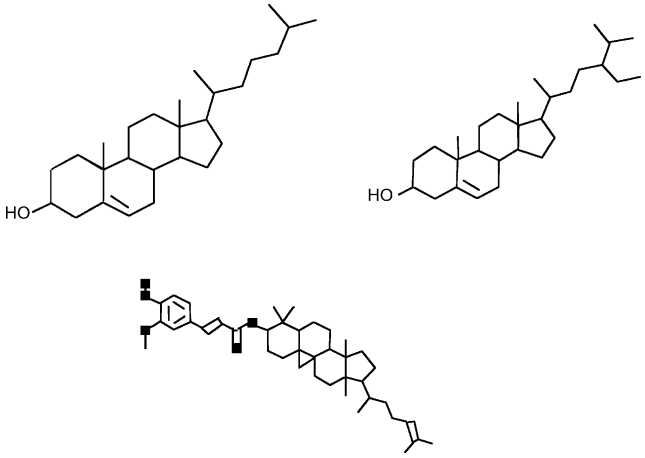

Fig. 1. Chemical structures of (clockwise from top) cholesterol, β-sitosterol, and cycloartenyl ferulate. (Figures were drawn courtesy of GIF/PNG-Creator for 2D Plots of Chemical Structures, http://www2.ccc.uni-erlangen.de/services/gifcreator/index.html).

40–50 g were fed powdered rodent chow for 1 week and then fed the experimental diets (Table 1) for 10 weeks. The dietary fat mixture used, i.e. 6% butterfat, 7% corn oil, and 2% fish oil, promoted increased LDL-C compared to chow or the same diet without fish oil (Fig. 2). A reference diet with additional corn oil substituted for fish oil was included. Increasing dietary cholesterol from 0.025% (natural concentrations found in butterfat and fish oil) to 0.05 and 0.20% resulted in an almost linear increase of LDL-C from about 125–325 mg/dl without changing high-density lipoprotein cholesterol (HDL-C) (Fig. 3). Plasma, was separated upon HPLC on a Superose HR6 column into three fractions that corresponded to VLDL, LDL, and HDL. Cholesterol in the separated fractions was determined simultaneously by a post-column reaction with a cholesterol reagent (Boehringer-Mannheim Diagnostics, Indianapolis, IN) and measurement of the absorption at 550 nm (German *et al*., 1996).

This study compared the cholesterol-lowering ability of 0.09% oryzanol from two commercial sources, Oryz3 and Oryz5, with that of 0.5% cholestyramine and 0.25% commercial phytosterol mixture which are well known cholesterol-lowering agents used to lower cholesterol in humans. The composition of the diet is shown in Table 1. The latter are as follows.

Plasma cholesterol lowering by oryzanol, cholestyramine and phytosterol

Plasma LDL-C declined about 25% upon feeding either 0.09% oryzanol or 0.25% phytosterol mixture, while it declined by 75% upon feeding 0.5% cholestyramine to hamsters fed the hypercholesterolemic fat mixture containing 0.05% cholesterol (Table 2). HDL-C was unchanged by oryzanol or phytosterol mixture feeding, but decreased by 20% when cholestyramine was fed. The ratio of LDL-C/HDL-C, or good to bad cholesterol, was improved by oryzanol or phytosterol feeding from 2.2 in the control to 1.3–1.5. Oryzanol feeding produced results similar to those of feeding a phytosterol mixture despite a 40% lower dose. Cholestyramine reduced LDL-C more than HDL-C, and the LDL-C/HDL-C ratio was extremely low, 0.45. Eliminating 2% fish oil from the diet decreased total plasma cholesterol 43% compared to the control, and HDL became the predominant lipoprotein carrier (59%) of cholesterol. The cholesterol and fish oil-fed hamster is an exquisitely sensitive bioassay, but its utility and sensitivity are reduced without fish oil, as higher concentrations of dietary cholesterol are required to induce elevated concentrations of plasma cholesterol which is often accompanied by increased liver weight.

Reduction of aortic fatty streaks by antioxidants

The high LDL-C in the butterfat and fish oil-fed hamster has been used to determine the antioxidant properties of vitamin E and catechin in vivo (Xu *et al*., 1998). The amounts of antioxidants fed were much lower than in studies using most other animal models and were comparable to amounts of vitamin E recommended for humans on a kilogram dry weight basis. Hamsters were fed diets containing low alpha-tocopherol (3 IU/kg diet), high alpha-tocopherol (30 IU/kg diet), or catechin (200 mg/kg diet). The diets were fed for 6 months. The aortas were removed, stained with Oil Red O, and analyzed using a microscopic image analysis system. Total and LDL-C was lowered by the high alpha-tocopherol diet compared to the low alpha-tocopherol diet. Aortic fatty streak area was 9.32±0.09, 6.57±0.86,

Table 1. Diet composition

Test ingredient and fat	Control	OZ3 or OZ5	Phytosterol	Cholestyramine	Reference diet
Cholestyramine	0	0	0	5	0
Oryzanol 3 or 5[a]	0	0.9	0	0	0
Phytosterol	0	0	2.5	0	0
Fish oil	20	20	20	20	0
Corn oil	70	70	70	70	90

Units are g/kg. Basal diet contained in g/kg, butterfat, anhydrous, 60; cellulose, 100; casein, 200; methionine, 3; choline bitartrate, 3; mineral mix, 35; vitamin mix, 10; and cholesterol, 0.75.

[a] Oryzanol 3 and 5 are two different commercial sources of oryzanol.

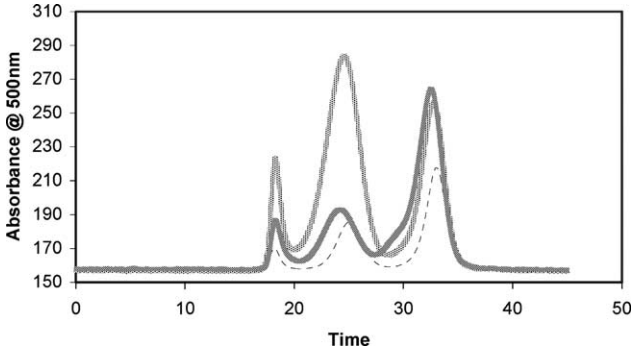

Fig. 2. HPLC chromatogram of hamster plasma separated by Superose HR-6 gel permeation column chromatography and post-column reaction with cholesterol reagent detected at 550 nm. Lipoprotein profile: fine black line, chow diet-fed hamster; heavy black line corn oil hyperlipidemic diet-fed hamster; gray line, fish oil hyperlipidemic diet.

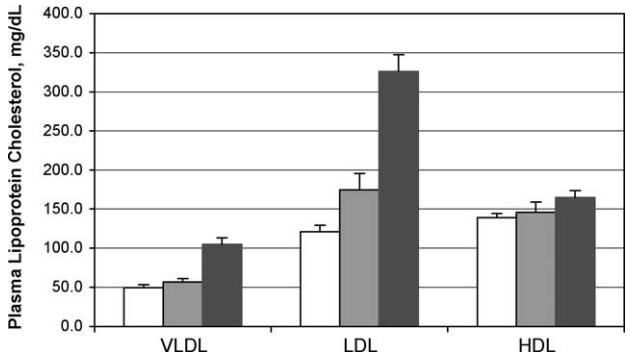

Fig. 3. Increasing cholesterol content of plasma lipoprotein fractions of hamsters fed different amounts of cholesterol: 0.025% cholesterol, clear bar; 0.05% cholesterol, gray bar; and 0.2% cholesterol, dark bar.

and 5.78±0.91% for tissue from hamsters fed the low alpha-tocopherol, catechin, and high alpha-tocopherol diets, respectively. Both antioxidant enriched diets reduced aortic streaks compared to the low alpha-tocopherol diet. Moreover, there was a linear correlation ($r=0.70$, $P<0.001$) between LDL-C concentration and the area percent of fatty streak of the aorta. Likewise, there was an inverse linear correlation ($r=-0.51$, $P<0.001$) between HDL-C concentration and the area percent of fatty streak of the aorta.

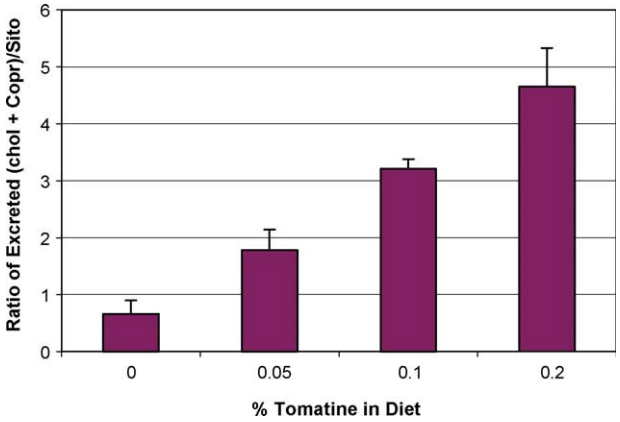

Fig. 4. Ratio of deuterated dietary cholesterol and sitostanol in feces of hamsters fed different amounts of tomatine.

Cholesterol excretion

The glycoalkaloid, tomatine, found in green but not red tomatoes, is known to tightly bind cholesterol in vitro. In order to determine if tomatine binds dietary cholesterol in vivo hamsters were fed different concentrations of tomatine and. d_6-cholesterol and d_4-sitostanol were added. Because sitostanol is poorly absorbed, it was assumed to be completely excreted. The ratio of d_6-cholesterol to d_4-sitostanol increased as dietary tomatine increased, indicating decreased absorption of dietary cholesterol in hypercholesterolemic hamsters fed 0.2% cholesterol Moreover, LDL-C was lowered when hamsters were fed 0.2% tomatine (Friedman, Fitch, & Yokoyama, 2000) (Fig. 4).

Conclusions

The hamster model with high concentrations of plasma LDL-C is conveniently and reproducibly induced by feeding a synthetic diet containing butterfat and fish oil. The increased LDL-C fraction is also useful for the induction of aortic fatty streaks and is positively and linearly correlated with aortic fatty streak formation. Hamsters fed this hypercholesterolemic diet appear to be more sensitive to decreases in the predominant LDL-C fraction by feeding cholesterol-reducing substances such as phytosterols, cholestyramine, and tomatine.

Table 2. Lipoprotein cholesterol, body weight, liver weight, and feed intake

Measurement	Control	OZ3	OZ5	Phytosterol	Cholestyramine	Reference diet
VLDL (mg/dl)	103±16	99±17	76±11	90±18	17±8	51±13
LDL (mg/dl)	369±32	284±13	280±17	264±18	87±4	101±11
HDL (mg/dl)	186±13	212±8	197±13	202±9	148±11	222±13
LDL/HDL	2.2±0.3	1.4±0.1	1.5±0.2	1.3±0.1	0.6±0.04	0.45
Final weight (g)	100±2	108±4	105±3	105±3	106±3	106±3
Weight gain (g)	53.9±2.6	60.0±4.1	59.4±3.5	58.0±2.8	61.4±2.6	60.4±2.5
Feed intake (g)	171±6	183±8	176±8	175±5	193±9	
Liver weight (g)	5.9±0.2	7.1±0.4	6.4±0.3	6.2±0.3	4.4±0.2	
Liver/body (%)	5.8±0.2	6.4±0.2	6.0±0.2	6.2±0.1	4.4±0.1	

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